10/620437
Patent #1-87

WHAT IS CLAIMED IS:

1. A process for obtaining a population of cells enriched in viable human liver cells, including hepatic stem/progenitor cells, comprising:

(a) digesting a whole human liver or resection thereof with a proteolytic enzyme preparation to provide a digested whole human liver or resection thereof;

(b) dissociating the digested whole human liver or resection thereof to provide a suspension of cells;

(c) adjusting the density of the medium in which the cells are suspended whereby at least two bands of cells separated by a density barrier are obtained upon centrifugation, at least one band of the at least two bands being of a lower density than another band of the at least two bands; and

(d) collecting the at least one band of lower density to obtain a population of cells enriched in viable human liver cells, including hepatic stem/progenitor cells.

2. The process of claim 1 in which the population of cells enriched in viable human liver cells further includes functional hepatocytes.

3. The process of claim 1 in which the population of cells enriched in viable human liver cells further includes functional biliary cells.

4. The process of claim 1 in which the population of cells enriched in viable human liver cells further includes functional hemopoietic cells.

5. The process of claim 1 in which step (a) includes:

(e) perfusing the whole human liver or resection thereof with a chelation buffer at approximately 37 °C for approximately 15 minutes;

(f) digesting the whole human liver or resection thereof with an enzyme preparation comprising collagenase and at least one other proteolytic enzyme at approximately 37°C for no longer than about 30 minutes to provide a digested liver; and

(g) perfusing the digested liver with collection buffer having a temperature of 4-15°C.

6. The process of claim 5 in which the enzyme preparation includes at least one neutral protease.

7. The process of claim 5 in which the enzyme preparation includes elastase.

1-28 I process
29-49 57 63, 71 73 I composition
50-56 70 II treat species disease #56
IV toxicity 64 65
V metabolism 66 67
VI device 68, 69
VII regeneration 74-77
VIII infection 78-85
IX vaccine 86 87

8. The process of claim 5 in which the enzyme preparation comprises LIBERASE™. 112nd

9. The process of claim 1 in which said dissociation includes mechanical dissociation.

10. The process of claim 9 in which said dissociation includes mechanical dissociation by cutting, raking, combing, or grating the liver.

11. The process of claim 1 in which step (c) includes:

(h) filtering the cell suspension to remove debris and cell aggregates;

(i) collecting the resulting filtered cell suspension in a first bag;

(j) optionally determining a concentration of cells in the filtered cell suspension;

(k) adjusting, if desired, the concentration of cells to provide a starting cell suspension;

(l) mixing an aliquot of the starting cell suspension with an equal volume of 25% iodixanol solution in a liquid medium to provide a mixture; and

(m) subjecting at least a portion of the mixture overlaid with a predetermined volume of the liquid medium to centrifugation to obtain at least one band enriched for viable human liver cells.

12. The process of claim 1 in which step (d) includes:

(n) collecting the *at least one* band into a container on ice;

(o) optionally determining viability and concentration of cells;

(p) washing the cells by centrifugation and resuspension in a cryopreservation buffer to obtain a final cell suspension;

(q) subjecting the final cell suspension to controlled rate freezing to provide a frozen cell suspension; and

(r) storing the frozen cell suspension in a liquid nitrogen freezer.

13. The process of claim 5 in which said collection buffer comprises RPMI 1640 medium with 10% human or bovine serum.

14. The process of claim 11 in which said filtering step includes passing said cell suspension through a filter cartridge.

15. The process of claim 11 in which said liquid medium comprises RPMI 1640 medium lacking phenol red.

16. The process of claim 11 in which said centrifugation is carried out for about 15 min at approximately 500 x g.

17. The process of claim 12 in which said container includes a collection bag.

18. The process of claim 12 in which the cryopreservation buffer comprises a mixture including Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, $H_2PO_4^-$, HCO_3^-, HEPES, lactobionate, sucrose, mannitol, glucose, Dextran-40, adenosine, glutathione, or combinations thereof.

19. The process of claim 18 in which the cryopreservation buffer further comprises serum and dimethylsulfoxide.

20. The process of claim 19 in which the mixture, serum and dimethylsulfoxide are present in a ratio of approximately 80:10:10 v/v/v.

21. The process of claim 19 in which the serum comprises human serum, bovine serum, or a combination thereof.

22. The process of claim 1 in which the density of the medium is adjusted by the use of an aqueous solution of iodixanol or iohexol.

23. The process of claim 22 in which the aqueous solution of iodixanol or iohexol comprises sterile 60% (w/v) iodixanol in water, and equivalent density of iohexol in water, or a combination thereof.

24. The process of claim 1 in which the density of the medium is adjusted by the use of an aqueous solution of a hydrophilic polymer of sucrose.

25. The process of claim 24 in which the aqueous solution of a hydrophilic polymer of sucrose comprises ficoll, ficoll plus diatrizoate with calcium EDTA, or a combination thereof.

26. The process of claim 1 in which the enriched population of cells includes hepatic progenitor/stem cells having a diameter in the range between 9 and 13 microns and which are positive for expression of EP-CAM, CD133, or both.

27. A process for obtaining an enriched population of viable human liver cells, which population of cells comprises functional hepatocytes and hepatic stem/progenitor cells, comprising:

(a) providing a whole human liver or resection thereof from neonatal, pediatric, juvenile, adult, or cadaver donor;

(b) perfusing the whole human liver or resection thereof with a chelation buffer at approximately 37 °C for approximately 15 minutes;

(c) digesting the whole human liver or resection thereof with an enzyme preparation comprising collagenase and elastase at 37 °C for no longer than about 30 minutes to provide a digested liver;

(d) perfusing the digested liver with chilled collection buffer;

(e) mechanically dissociating the digested liver to provide a cell suspension;

(f) passing the cell suspension through a filter cartridge to remove debris and cell aggregates;

(g) collecting the resulting filtered cell suspension in a first bag;

(h) optionally determining viability and concentration of cells in the filtered cell suspension;

(i) adjusting the concentration to about 25 million cells per mL to provide a starting cell suspension;

(j) mixing in a second bag an aliquot (250 mL) of the starting cell suspension with an equal volume of 25% iodixanol (OptiPrep™) solution in RPMI 1640 medium lacking phenol red;

(k) subjecting (500 mL) of the resulting mixture overlaid with a predetermined volume (60 mL) of RPMI 1640 medium lacking phenol red to centrifugation on a COBE™ 2991 Cell Processor (15 min at 2000 rpm, ca. 500 x g) to obtain at least one band enriched for viable cells;

(l) collecting the at least one band into a third bag on ice;

(m) optionally, determining viability and concentration of cells in the third bag;

(n) washing the cells in the third bag by centrifugation and resuspension in cryopreservation buffer to obtain a final cell suspension;

(o) subjecting the final cell suspension to controlled rate freezing to provide a frozen cell suspension;

(p) storing the frozen cell suspension in a liquid nitrogen freezer.

28. The process of claim 27 in which the enriched population of cells is enriched in hepatic progenitor/stem cells having a diameter in the range between about 9 and about 13 microns and which are positive for expression of EP-CAM, CD133, or both.

29. A composition comprising a population of cells enriched in viable, functional liver cells, including hepatic stem/progenitor cells.

30. The composition of claim 29 in which the liver cells are human liver cells, or mammalian liver cells.

31. The composition of claim 30 which further comprise hepatocytes, biliary cells, hemopoietic cells, or combinations thereof.

32. The composition of claim 29 in which the liver cells comprise cells which are positive for expression of EP-CAM, CD133, or both.

33. The composition of claim 29 in which the liver cells comprise stem/progenitor cells of approximately 9 to 13 microns in diameter.

34. A composition comprising a population of liver cells enriched, relative to a crude suspension of cells obtained from liver, in viable, functional hepatocytes and hepatic stem/progenitor cells.

35. The composition of claim 34 in which the liver cells are human liver cells.

36. The composition of claim 35 in which the population of liver cells further comprises biliary cells, which are positive for expression of cytokeratin 19 (CK19) and negative for expression of albumin.

37. The composition of claim 34 in which the stem/progenitor cells are positive for the expression of EP-CAM, CD133, or both.

38. The composition of claim 36 in which the population further comprises monocytes/macrophage lineage cells (e.g., Kupffer cells), lymphocytes (e.g., T lymphocytes), endothelial cells, or combinations thereof.

39. The composition of claim 34 in which the population of liver cells is depleted of cells of the immune system.

40. The composition of claim 39 in which the population of liver cells is depleted of cells of the immune system by use of negative immunoselection.

41. The composition of claim 40 in which the negative immunoselection utilizes CD45, CD3, CD11b, or CD14 depletion, or combinations thereof, to remove hemopoietic cells.

42. The composition of claim 39 in which the cells of the immune system which are depleted include monocytes/macrophage lineage cells (e.g., Kupffer cells), lymphocytes (e.g., T lymphocytes), or both.

43. The composition of claim 34 in which the donor liver has been subjected to a period of warm ischemia.

44. The composition of claim 34 in which the liver cells are obtained from an asystolic donor.

45. The composition of claim 34 in which at least 75% of the population of liver cells comprises hepatocytes and hepatic progenitor/stem cells.

46. A composition comprising a population of liver cells enriched in viable, functional biliary cells and hepatic stem/progenitor cells.

47. The composition of claim 46 in which the liver cells are human liver cells, porcine liver cells, or mixtures thereof.

48. The composition of claim 46 in which the liver cells are obtained from an asystolic donor or have been subjected to a period of warm ischemia.

49. The composition of claim 46 in which the biliary cells are positive for expression of CK19 and negative for expression of albumin.

50. A method of treating liver disease comprising administering an effective amount of a population of cells enriched in viable, functional liver cells, including hepatic stem/progenitor cells.

51. The method of claim 50 in which administration is effected by introduction through a splenic artery or portal vein.

52. The method of claim 50 in which administration is effected by introduction directly into the liver pulp.

53. The method of claim 50 in which administration is effected by introduction under the liver capsule.

54. The method of claim 50 in which administration is effected by introduction directly into the spleen.

55. The method of claim 50 in which introduction is by infusion or injection.

56. The method of claim 50 in which the liver disease includes hepatitis, cirrhosis, inborn errors of metabolism, acute liver failure, acute liver infections, acute chemical toxicity, chronic liver failure, cholangiocitis, biliary cirrhosis, Alagille syndrome, alpha-1-antitrypsin deficiency, autoimmune hepatitis, biliary atresia, cancer of the liver, cystic disease of the liver, fatty liver, galactosemia, gallstones, Gilbert's syndrome, hemochromatosis, hepatitis A, hepatitis B, hepatitis C, and other hepatitis viral infections, poryphyria, primary sclerosing cholangitis, Reye's syndrome, sarcoidosis, tyrosinemia, type 1 glycogen storage disease, or Wilson's disease.

57. A pharmaceutical composition comprising a population of liver cells enriched in viable, functional liver cells, including hepatic stem/progenitor cells and a pharmaceutically acceptable carrier.

58. The composition of claim 57 in which the liver cells are human liver cells, porcine liver cells, or mixtures thereof.

59. The composition of claim 57 in which the liver cells are obtained from an asystolic donor or have been subjected to a period of warm ischemia.

60. The composition of claim 57 in which the stem/progenitor cells are positive for expression of EP-CAM, CD133, or both.

61. The composition of claim 57 in which the stem/progenitor cells are approximately 9 to 13 microns in diameter.

62 The composition of claim 57 in which the pharmaceutically acceptable carrier includes HYPOTHERMOSOL™.

63. The composition of claim 57 in which the pharmaceutically acceptable carrier further includes human serum and dimethylsulfoxide.

64. A method of conducting *in vitro* toxicity testing comprising:

(i) exposing to a test agent a population of liver cells enriched in viable, functional liver cells, including hepatic stem/progenitor cells, and

(ii) observing at least one effect, if any, of the test agent on the population of liver cells.

65. The method of claim 64 in which the at least one effect includes an effect on cell viability, cell function, or both.

66. A method of conducting *in vitro* drug metabolism studies comprising:

(i) exposing a population of liver cells enriched in viable, functional liver cells, including hepatic stem/progenitor cells, to a test agent, and

(ii) observing at least one change, if any, involving the test agent after a predetermined test period.

67. The method of claim 66 in which the at least one change includes a change in the structure, concentration, or both of the test agent.

68. A liver assist device comprising a housing harboring a population of cells enriched in viable, functional liver cells, including hepatic stem/progenitor cells.

69. The device of claim 68 in which the liver cells are depleted of immune system cells.

70. A method for treating errors of gene expression comprising:

(i) introducing into a population of human liver cells including viable, functional hepatic stem/progenitor cells a functional copy of a gene to provide a transformed population; and

(ii) introducing into a patient's liver, which patient is in need of the functional copy of the gene, at least a portion of the transformed population.

71. A composition for treating errors of gene expression comprising a transformed population of human liver cells including viable, functional hepatic stem/progenitor cells into which a functional copy of a gene has been introduced.

72. A pharmaceutical composition for treating errors of gene expression comprising a population of human liver cells including viable, functional hepatic

stem/progenitor cells into which a functional copy of a gene has been introduced and a pharmaceutically acceptable carrier.

73. The composition of claim 72 in which the pharmaceutically acceptable carrier includes a mixture including Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, $H_2PO_4^-$, HCO_3^-, HEPES, lactobionate, sucrose, mannitol, glucose, Dextran-40, adenosine, glutathione, or combinations thereof.

74. A method for enhancing the regeneration of an injured or diseased liver comprising administering into the liver an effective amount of a population of human liver cells enriched in viable, functional liver cells, including hepatic stem/progenitor cells.

75. The method of claim 74 in which administration is effected by introduction through a splenic artery or portal vein, directly into the liver pulp, under the liver capsule, or combinations thereof.

76. The method of claim 74 in which administration is effected by introduction directly into the spleen.

77. The method of claim 74 in which introduction is by infusion or injection.

78. A method of conducting testing for efficacious agents for treating liver infections comprising (i) infecting with an infectious agent of interest a population of human liver cells enriched in viable, functional liver cells, including hepatic stem/progenitor cells to provide an infected population, (ii) exposing the infected population to a predetermined amount of test agent, and (iii) observing effects, if any, of the exposure on the infected population.

79. The method of claim 78 in which the infectious agent includes a microorganism.

80. The method of claim 78 in which the infectious agent includes one or more viruses, bacteria, fungi, or combinations thereof.

81. The method of claim 80 in which the observed effects include effects on viral replication of a viral infectious agent.

82. The method of claim 81 in which the viral infectious agent includes a hepatitis virus.

83. A method of producing a protein of interest comprising introducing into a population of liver cells including hepatic progenitor/stem cells a functional gene encoding a protein of interest, incubating the population of liver cells under conditions effective for transcription, translation, and optionally post-translational modification to take place, and harvesting the protein of interest.

84. The method of claim 83 in which the liver cells are human liver cells.

85. The method of claim 83 in which the protein of interest comprises a vaccine antigen.

86. A method of producing a vaccine comprising introducing into a population of liver cells including hepatic progenitor/stem cells a recombinant virus or virion particle capable of infecting at least some members of the population of liver cells causing the infected members to express an antigen such that an immune response is elicited from a subject seeking to be immunized against future exposure to an infectious agent associated with the antigen upon introduction of the infected members of the population into the subject.

87. The method of claim 86 in which a cell-based immune response is elicited.